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## Tuning the 'spontaneous' formation kinetics of caffeine:malonic acid co-crystals

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It has previously been reported that the caffeine:malonic acid co-crystal system forms spontaneously on the contact of the two materials. Here we studied the crystal growth in this system to rationally define the role of water plays, thus enabling us to monitor the conversion by solid-state NMR and control the kinetics of 'spontaneous' co-crystal production.

Caffeine (1,3,7-trimethyl-2,6-purinedione) is the world's most widely used psychoactive drug. It is cleared rapidly from the body with a half-life of around 5 hours (for the average cup of fresh coffee).<sup>1</sup> As well as being a good 'model' drug caffeine is also included in many pharmaceutical preparations, so there is considerable pharmaceutical interest in the material properties of its crystalline state. It has a two polymorphic forms and a stable non-stoichiometric hydrate.<sup>2</sup> With the  $\beta$ -anhydrous form being the most stable in 'temperate' atmospheric conditions of around 40% relative humidity and 25°C.<sup>3</sup>

Co-crystals are most widely defined as: 'solids that are crystalline single phase materials composed of two or more different molecular and/or ionic compounds generally in a stoichiometric ratio which are neither solvates nor simple salts'.<sup>4</sup> Co-crystals, along with salts and amorphous forms, represent a pathway to the modification of the materials properties of a molecule and are extensively used in the pharmaceutical arena (for drug delivery) as well as in others such as agrochemicals.<sup>5,6</sup>

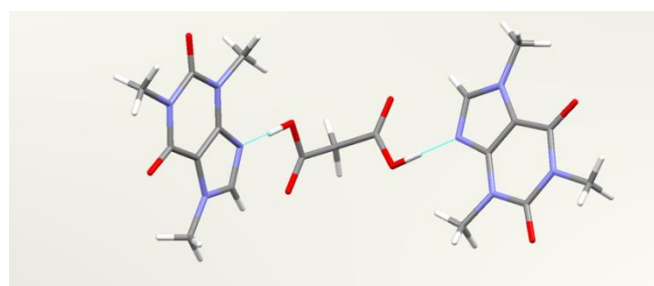


Figure 1. Caffeine (CA) and malonic acid (MA) co-crystal interactions (CSD REF-GANYAW)

A total of 109 co-crystals of caffeine (Caf hereafter) are present in the current edition of the Cambridge Structural Database (CSD).<sup>7</sup> Pharmaceutical Caf co-crystals were first reported in 1980,<sup>2,8,9</sup> and their properties have subsequently been shown to be beneficial both in terms of hydration behaviour<sup>2</sup> and compression potential.<sup>10</sup>

Production of these binary adducts is achieved through the application of a number of differing approaches; mechano-chemical/solvent drop grinding,<sup>2,11</sup> solution production,<sup>9</sup> and heteronuclear seeding.<sup>12</sup> The Caf:malonic acid 2:1 co-crystal (Figure 1) however has been reported to form spontaneously, which sets it apart from other Caf co-crystals. This was first seen on reduction of its particle size,<sup>13,14</sup> and has subsequently been studied by solid-state NMR.<sup>15</sup> Neither study however yielded a firm conclusion as to a specific molecular-level growth mechanism for these forms. As malonic acid (MA hereafter) is a hygroscopic co-former<sup>16</sup> and Caf possesses a well-known hydrate we set out to investigate the impact of water on the formation of this co-crystal, to enable a better understanding of co-crystal growth with Caf.

Previous reports of spontaneous formation of the co-crystal have focused on the particle size, which has been shown to have a significant impact on the rate of conversion from the individual components to the co-crystal.<sup>14</sup> Here the dry starting materials were ball-milled to consistent particle size before all further investigations to remove the influence of this effect. (Fig. S1, S2 ESI†). The Caf hydrate material dehydrated on milling, so was used as delivered by a 48-hour slurry in water.

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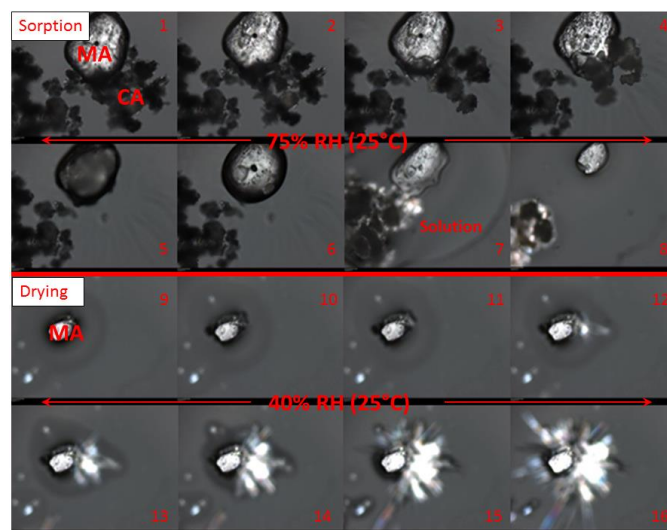
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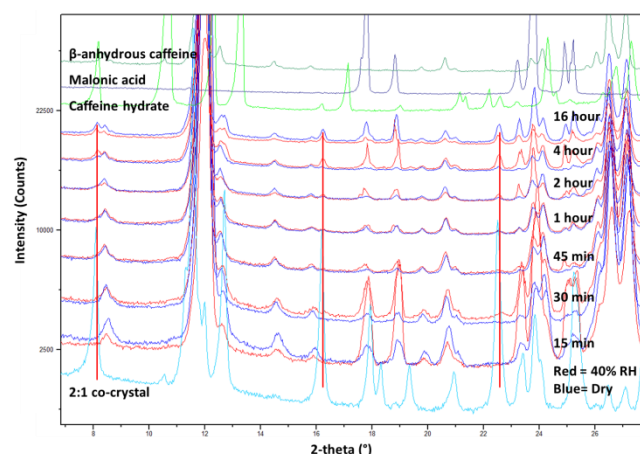
Milling can induce amorphicity in materials, with passage through a transient amorphous phase proposed as a potential mechanism for growth in this co-crystal system.<sup>14</sup> To understand the potential of co-crystal conversion being due to this, the amorphous behaviours of the starting components were explored by modulated DSC. (Fig. S3, S4 ESI†) MA recrystallized quickly on cooling, making it impossible to determine its glass transition in a heat cool heat experiment and further attempts to stabilise the glass were unsuccessful. This correlates with the lack of literature data on its glass transition and would suggest that it is a class I material with respect to its amorphous stability.<sup>17</sup> Caf was seen to have a  $T_g$  of around 49.3°C (322.3K) when prepared in the DSC pan at a cooling and heating rate of 3K/min. This was higher than has previously been reported,<sup>18</sup> but it should be noted that this was recorded at a different heating rate. From these results it is clear that no annealing was necessary to ensure MA surface crystallinity, but to ensure Caf crystallinity a 48 hour period of annealing at 50°C was used for the residual anhydrous material. The hydrate was not subjected to these conditions as it is not stable under them; converting back to the stable anhydrous form. The hydrate of Caf was also seen to revert to the  $\beta$ -form in dynamic vapour sorption (DVS) experiments. (Fig. S5-11 ESI†) These data correlated with previous reports on the hydrate, displaying the need of higher than 90% relative humidity (RH) at 25°C to return it to the hydrated state. The  $\beta$ -form of Caf is non-hygroscopic, and neither are adipic and succinic acid; both of which have displayed difficulty in co-crystal formation in the past.<sup>8,9</sup> MA however is readily hygroscopic above a relative humidity threshold of 70% RH (25°C), but loss of moisture once absorbed is not seen until humidities below 10% RH (25°C). So if the sample is exposed to moisture it will not readily lose it. With this in mind samples of MA and Caf were exposed to a variety of humidities under polarised microscopy within a chamber containing a saturated salt solution (Fig. S12-18 ESI†). Results from the 75% RH (25°C) condition can be seen in Figure 2, in this instance a large malonic acid crystal, rather than milled material, was used for visual clarity. This showed partial deliquescence of the MA, which in turn dissolved the Caf. This state could be maintained for up to 7 days with no change seen under optical microscopy. On drying at ambient humidity (40% RH 25°C) new crystal growth was seen. From the microscopy experiments alone it was not possible to assign the new growth to a particular phase (i.e. the co-crystal) with certainty, although the transitions recorded suggest growth of this phase.. Similar experiments conducted at 40% relative humidity show no visible conversion after 7 days. Experiments at 75% RH were also conducted with adipic and succinic acid, which similarly displayed no conversion from the starting materials (Fig. S12-18 ESI†). To avoid the uncertainty of phase identification in microscopy, and to map the crystal growth process, samples were prepared at the 2 gram scale. This was done using a 2:1 Caf:MA mix and the molar proportions of pure water seen to cause deliquescence in malonic acid from the DVS experiments.



**Figure 2.** Production of a new phase, thought to be the co-crystal, from the absorption of atmospheric water. MA= Malonic acid, CA= Caffeine. Each picture is taken at a 1 minute interval with 1-8 sorption and 9-16 drying.

These were then allowed to dry in ambient conditions, with the results analysed by powder X-ray diffraction. When the  $\beta$ -anhydrous Caf was used samples initially completely dissolved, with the first solid phase to be produced from the solution being the caffeine hydrate, which rapidly converts to the co-crystal (Figure S21-23 ESI†). When the caffeine hydrate is used as the starting material complete dissolution did not occur, with direct conversion from the hydrate to the co-crystal, thus giving insight into the ternary phase behaviours of this system. Finally in order to determine the long term impact of water sorption on conversion kinetics stoichiometric mixtures of MA and Caf were stored at 11, 60 and 75% RH respectively for 9 months. On analysis there was no conversion seen in the 11% RH environment, little conversion seen at 60% RH and full conversion at 75% RH. These results show that macroscopically the 'spontaneous' mechanism of co-crystal growth is through hygroscopicity and deliquescence, followed by dissolution and then solution crystal growth at a surface.

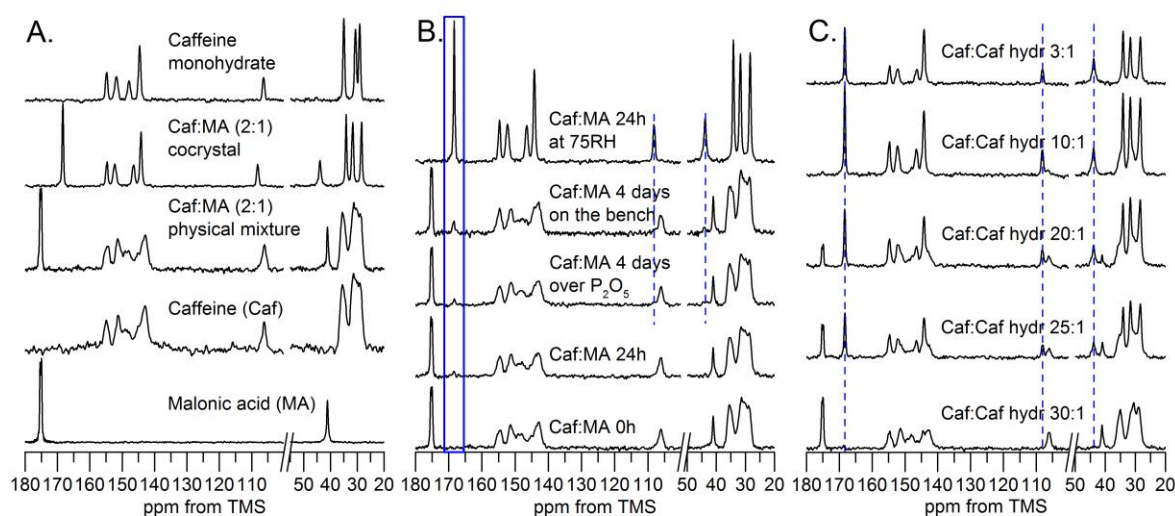
Having shown that hygroscopicity has a significant impact on the spontaneous formation of the co-crystal, in order to determine if hydration conditions impact on the rate of conversion by mechanical grinding samples were prepared in a 'dry' environment. To achieve this, a drying oven at 50°C and 5% RH was used for all manipulation of the samples, which were independently prepared for a number of time points and then sealed. Dry samples were then compared against those stored at ambient humidity with respect to the rate of conversion to the co-crystal. As can be seen in Figure 3 both the dry and 'wet' samples (both of the same particle size) displayed no difference in the rate of conversion to the co-crystal; both displaying characteristic X-ray diffraction peaks of the co-crystal phase at 1 hour.



**Figure 3.** Powder X-ray diffraction of ground samples displaying characteristic peaks of the co-crystal from 1 hour onwards in both dry and 40% RH samples.

$^1\text{H}$ - $^{13}\text{C}$  CP/MAS solid-state NMR spectra of Caf monohydrate and Caf:MA co-crystal shows one sharp peak per carbon atom indicating the presence of one molecule in the asymmetric unit. Similarly,  $^1\text{H}$ - $^{13}\text{C}$  CP/MAS solid-state NMR spectrum of malonic acid shows two carbonyl peaks at 175.3 and 174.9 ppm and one aliphatic peak at 41.1 ppm. It is worth noticing that both carbonyl sites of MA in Caf:MA co-crystal are in the same local environment leading to single peak at 168.3 ppm in the  $^{13}\text{C}$  spectrum. The  $^{13}\text{C}$  spectrum of anhydrous caffeine shows broadening of the peaks which is related to previously described local disorder in caffeine crystals (Figure 4A),<sup>19,20</sup> the detailed assignment of the spectra is presented in the ESI (Fig. S24<sup>†</sup>). The disorder in caffeine crystals and further 'ordering' of caffeine molecules in Caf:MA co-crystal can be observed in high resolution  $^1\text{H}$  CRAMPS spectra. Only two broad peaks are observed in  $^1\text{H}$  spectrum of anhydrous caffeine, while all four proton sites of Caf can be distinguished in Caf:MA co-crystal (Fig. S25 ESI<sup>†</sup>). Due to clear differences in the position of the carbonyl peaks of pure MA and its co-

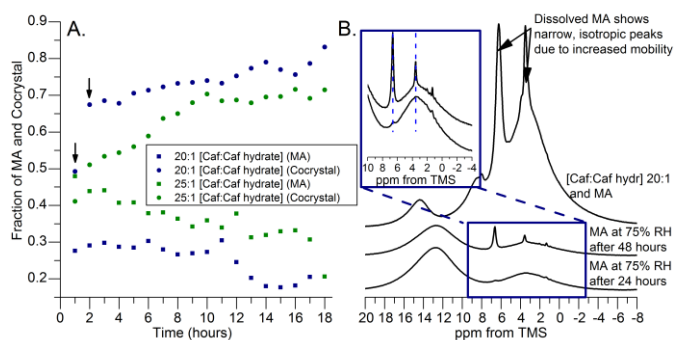
crystal with Caf in  $^{13}\text{C}$  spectra we were able to semi-quantitatively monitor the transition of the Caf/MA mixture to the Caf:MA co-crystal. As the spontaneity of the formation of Caf:MA co-crystal is strongly dependent on the atmospheric conditions, we assessed the effect of water on the transformation process in a controllable manner. The kinetics of the reaction in relation to water content was investigated by solid-state NMR using an approach previously described by Mandala et al.<sup>15</sup> The solid-state NMR spectra show very little conversion of dry Caf/MA mixture in 2:1 stoichiometric ratio to Caf:MA co-crystal after either four days of storage in dry conditions or four days storage on the bench at ca. 45% RH (Fig. 4B and Fig. S25-27 ESI<sup>†</sup>). When the same material was transferred to the high humidity chamber (75% RH) full conversion of Caf/MA mixture to Caf:MA co-crystal was observed after 24 hours (Figure 4B). There are several examples illustrating that an increased humidity or vapour pressure may affect the initial rate of formation of co-crystals.<sup>16,21</sup> Our recent work on co-crystals of benzoic acid and isoniazid demonstrated an increased rate of co-crystallisation upon the increased humidity and highlighted the importance of changes of surface mesostructure for such processes.<sup>22</sup> To further investigate the effect of water on conversion of Caf/MA mixture to Caf:MA co-crystal, the content of water within the sample was changed in controllable manner by mixing anhydrous caffeine with caffeine monohydrate in molar ratios ranging from 3:1 to 30:1 [Caf:Caf monohydrate], so that the Caf/MA stoichiometry is not affected. The mixture of both caffeine phases was subsequently vortexed with MA in order to yield Caf:MA co-crystal. The spectra of the materials with different water contents recorded within the first hour of the reaction give a clear indication that the higher the water content within the material the higher the initial rate of Caf/MA mixture to Caf:MA co-crystal transformation (Figure 4C).



**Figure 4.** A.  $^1\text{H}$ - $^{13}\text{C}$  solid-state NMR spectra of malonic acid, caffeine anhydrous, caffeine monohydrate, Caf:MA physical mixture and Caf:MA co-crystal. B. Transition of Caf/MA dry mixture to Caf:MA co-crystal in dry and humid conditions. C.  $^1\text{H}$ - $^{13}\text{C}$  CP/MAS NMR spectra showing the effect of water on the rate of formation of Caf:MA co-crystal.



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**Figure 5.** A. Kinetics of Caf:MA co-crystallisation process for mixtures of Caf:Caf hydrate with 20:1 and 25:1 [Caf:Caf hydrate] molar ratio. B.  $^1\text{H}$  solid-state NMR spectra of MA after 24 and 48 hours storage at 75% RH and [Caf:Caf hydr] 20:1 mixture with MA.

The kinetics of the co-crystallisation of two reaction mixtures using [Caf:Caf hydrate] of 20:1 and 25:1 was investigated in more details in a series of  $^1\text{H}$ - $^{13}\text{C}$  CP/MAS NMR experiments during first 18 hours of the transformation. The changes in the intensity of the carbonyl peaks of MA in a pure form and MA in the co-crystal were used to monitor the kinetics. The mixture with the smaller amount of water [Caf:Caf hydr] 25:1 shows a typical behaviour in which a decrease of the content of MA is related to increase of Caf:MA co-crystal content (Figure 5A – green marks). That is contrasted with the kinetics in the system with the larger water content [Caf:Caf hydr] 20:1. After first hour of the experiment it can be observed that only ca. 80% of the total malonic acid signal is observed. Furthermore, large increase in the co-crystal content is observed after second hour of the experiment, while the MA content stays constant (Figure 5A – blue marks). Such a behaviour of the mixture with the higher water content is corroborated by  $^1\text{H}$  solid-state NMR experiments and can be explained as follows. In first hour of the experiment the MA dissolves in the water, which is immediately released from the Caf monohydrate on contact with MA. This can be seen to occur visually (Fig. S28 ESI $^{+}$ ). The dissolved species of MA cannot be observed in the  $^1\text{H}$ - $^{13}\text{C}$  CP/MAS NMR spectrum due to their high mobility and insufficient transfer of magnetisation during cross polarisation leading to decrease of the total NMR signal. As the dissolved MA can facilitate the transition towards Caf:MA co-crystal, a significant increase in the co-crystal content can be observed after second hour of the experiment. This hypothesis is in excellent agreement with  $^1\text{H}$  NMR data. Deliquescence of the MA crystal is observed after storage at higher than 60% RH, which can be related to dissolving of the MA molecules at the crystal surface. Since it is possible to distinguish molecules in two different environments (motional regimes) using NMR i.e. species in crystalline, rigid component and dissolved species at the crystal surface, we observed growth of two narrow,

isotropic peaks of dissolved MA after storage at 75% RH for 48 hours (Figure 5B). Similar peaks can be observed in  $^1\text{H}$  solid-state NMR spectrum of [Caf:Caf hydr] 20:1 mixture with MA, proving presence of highly mobile dissolved species of MA in the crystallisation mixture.

In this work we have determined mechanistic insight into the Caf:MA co-crystal formation process. We have been able to tune the 'spontaneous' conversion rate from starting materials to co-crystal by tailored moisture addition. As such we are able to conclude that moisture sorption is a significant method by which growth of the Caf:MA co-crystal progresses. Moreover it is clear that systems which lack the hygroscopicity displayed by MA do not display this growth mechanism.

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